# Establishing Adverse Outcome Pathways of Thyroid Hormone Disruption in an Amphibian Model

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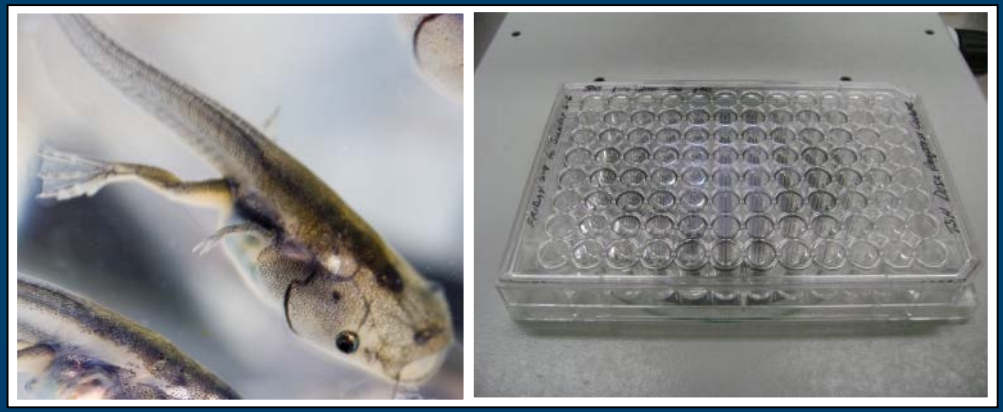

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# Working within an AOP Framework

- How many AOPs are there?
- Which AOPs should be focused on?
- How should interactions among AOPs be assessed?
- How conserved are AOPs across species?
- How detailed do AOPs need to be?
- What is the best approach for linking exposure (ADME) to AOPs?
- How can AOPs be catalogued/depicted for use by risk assessors?

# Which AOPs to focus on ?

- Safe Drinking Water Act & Food Quality Protection Act mandate that USEPA assess chemicals for endocrine activity

  - Many chemicals with limited or no information on thyroid activity

  - Need methods for prioritization for testing

# Objectives

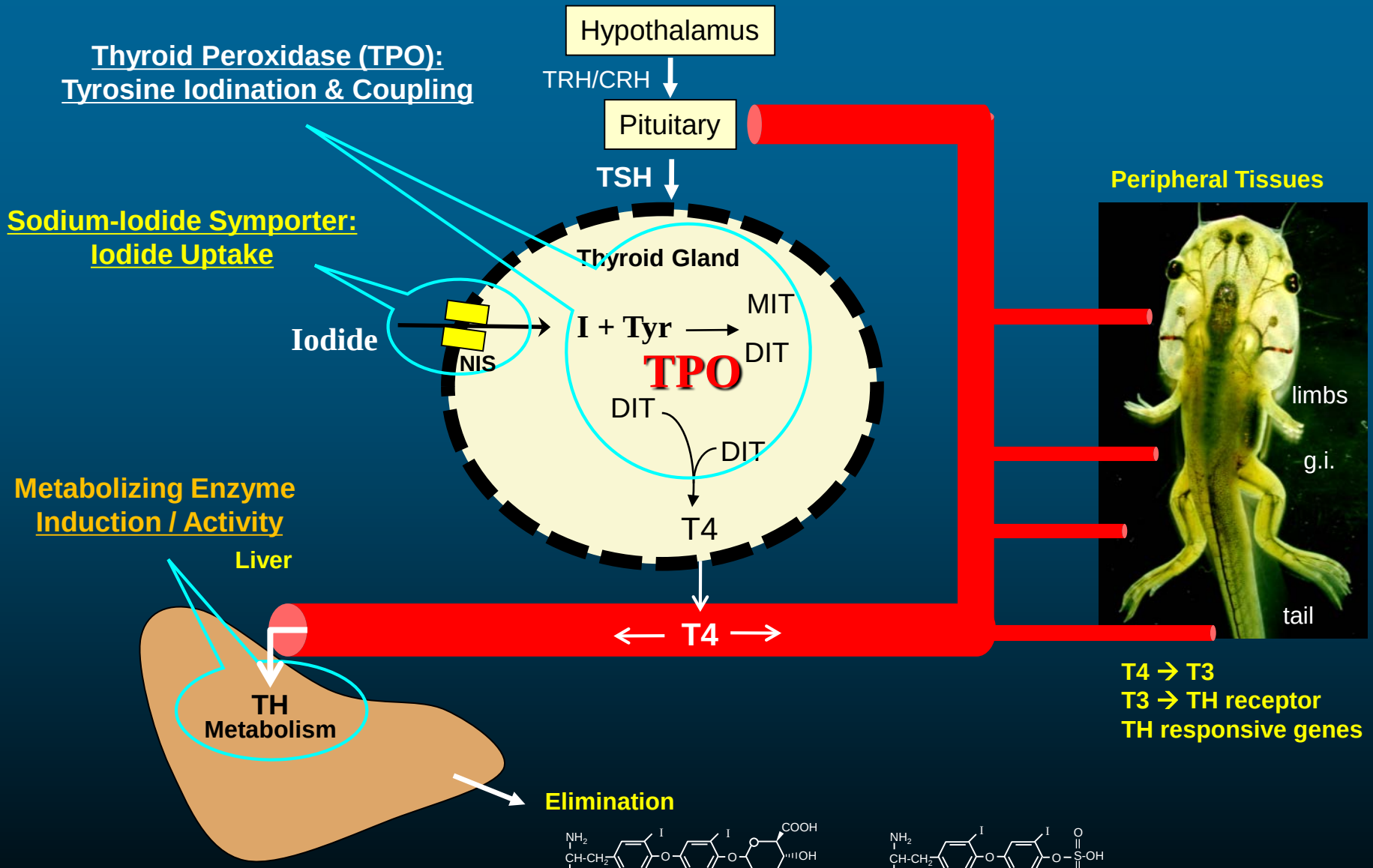
- Develop higher throughput, mechanism-based, predictive tools for disruption of thyroid hormone
- Support the development of chemical structure activity relationship (QSAR) models for thyroid hormone disrupting activity = predictive models
- Develop and conduct assays in the context of an AOP

## What endpoint to focus on:

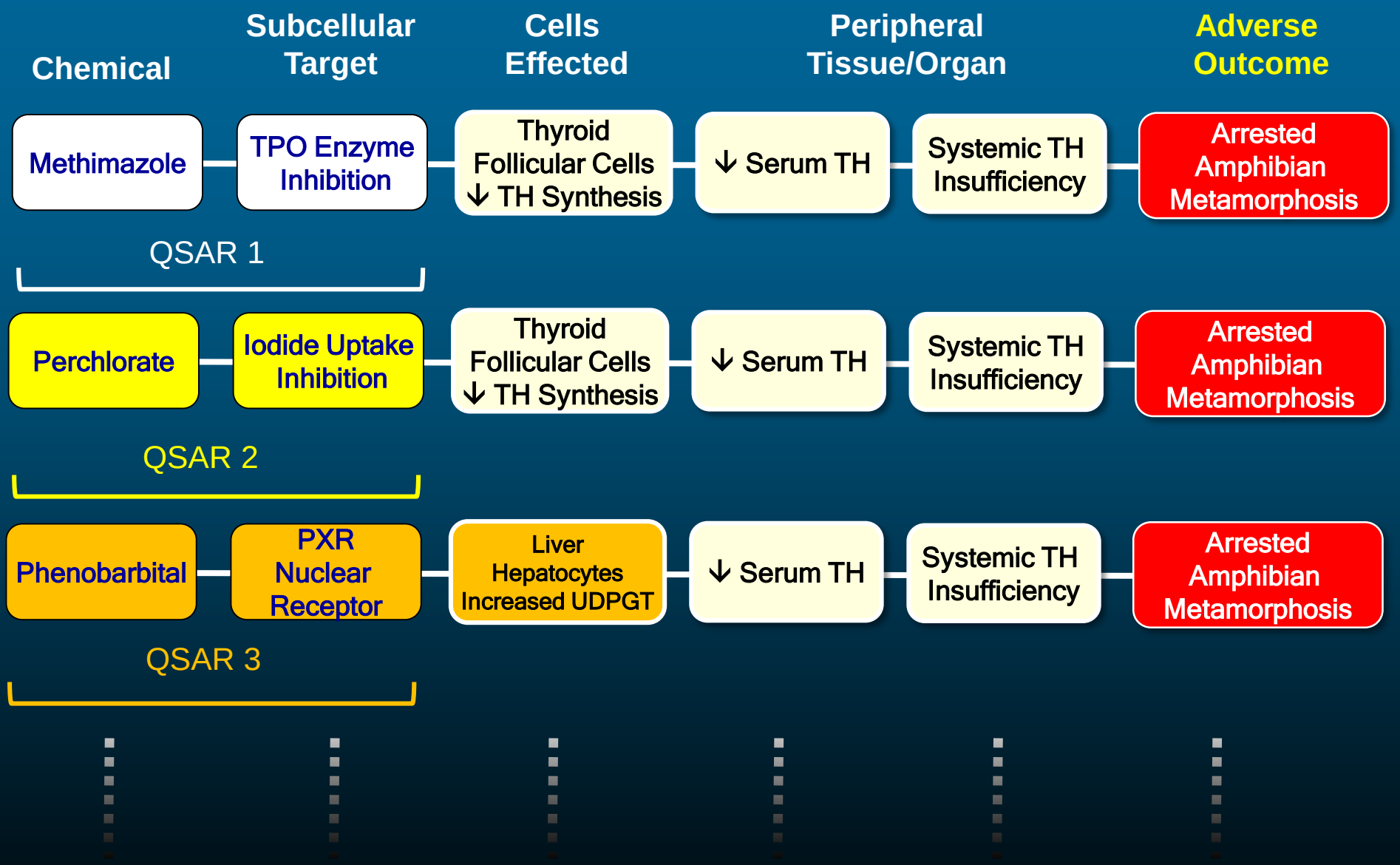
Disruption of maintenance of adequate thyroid hormone can affect multiple processes:

- Growth:
- Metabolism:
- Reproduction:
- Neurodevelopment:
- Amphibian Metamorphosis:

# Components of the System of Interest



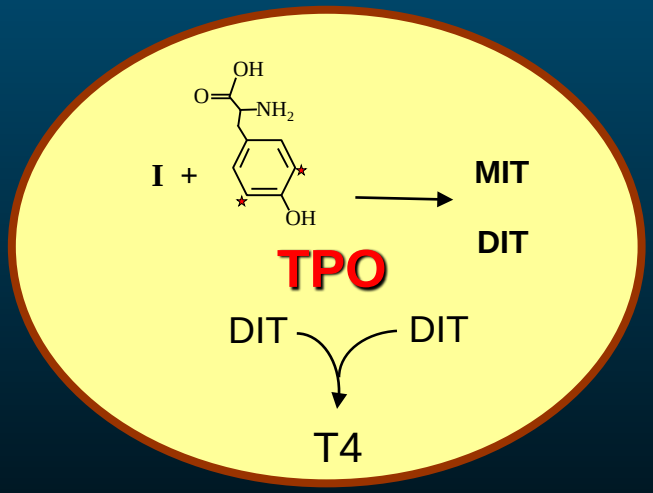
# Adverse Outcome Pathways for Thyroid Hormone Disruption

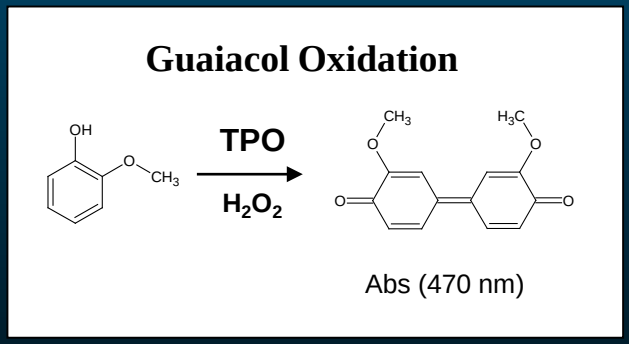


# Selection of a Molecular Initiating Event

## *Thyroid Peroxidase Inhibition*

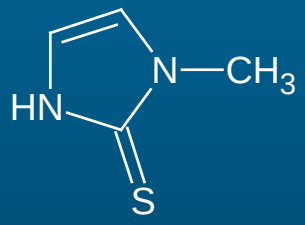
- Relatively Simple In Vitro Assay
- Prepare microsomes from thyroid glands (porcine)
- In Vitro Assay:  96-well plate
- Colorimetric Endpoint



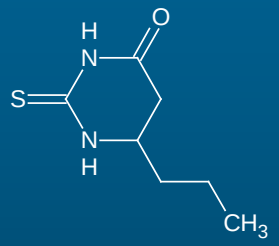



# Selection of Chemicals to Test in the In Vitro Assays

- **Test chemicals based upon <u>structural similarity to known active chemicals : model pharmaceuticals</u>**

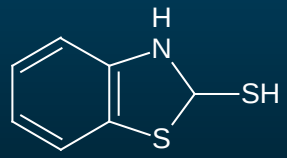



methimazole

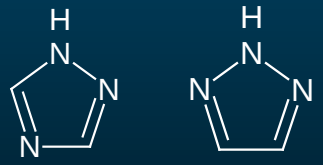



PTU

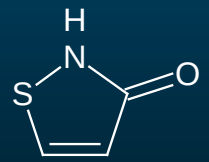
## Test chemical classes on EPA inventories




thiazoles and benzothiazoles




triazoles

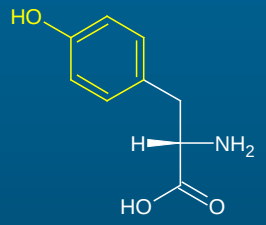



isothiazolinones

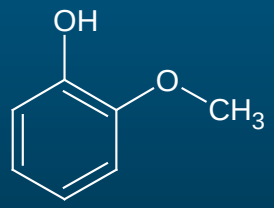

triazines

# Selection of Chemicals to Test in the In Vitro Assays

- **Test additional chemicals based upon <u>structural similarity to known TPO substrates: potential competitive inhibitors</u>**
  - Endogenous substrate: tyrosine

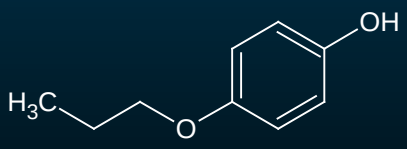



  - TPO enzyme assay substrate: guaiacol (*o*-methoxyphenol)

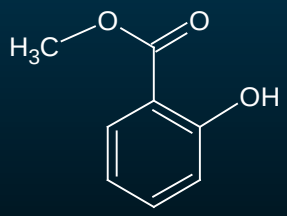



**Test Chemical Classes on EPA Inventories**

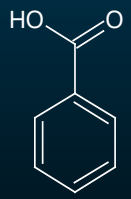
alkoxyphenols



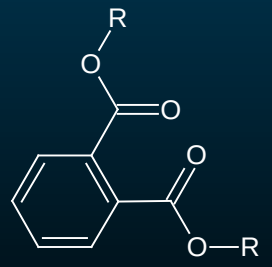

salicylates



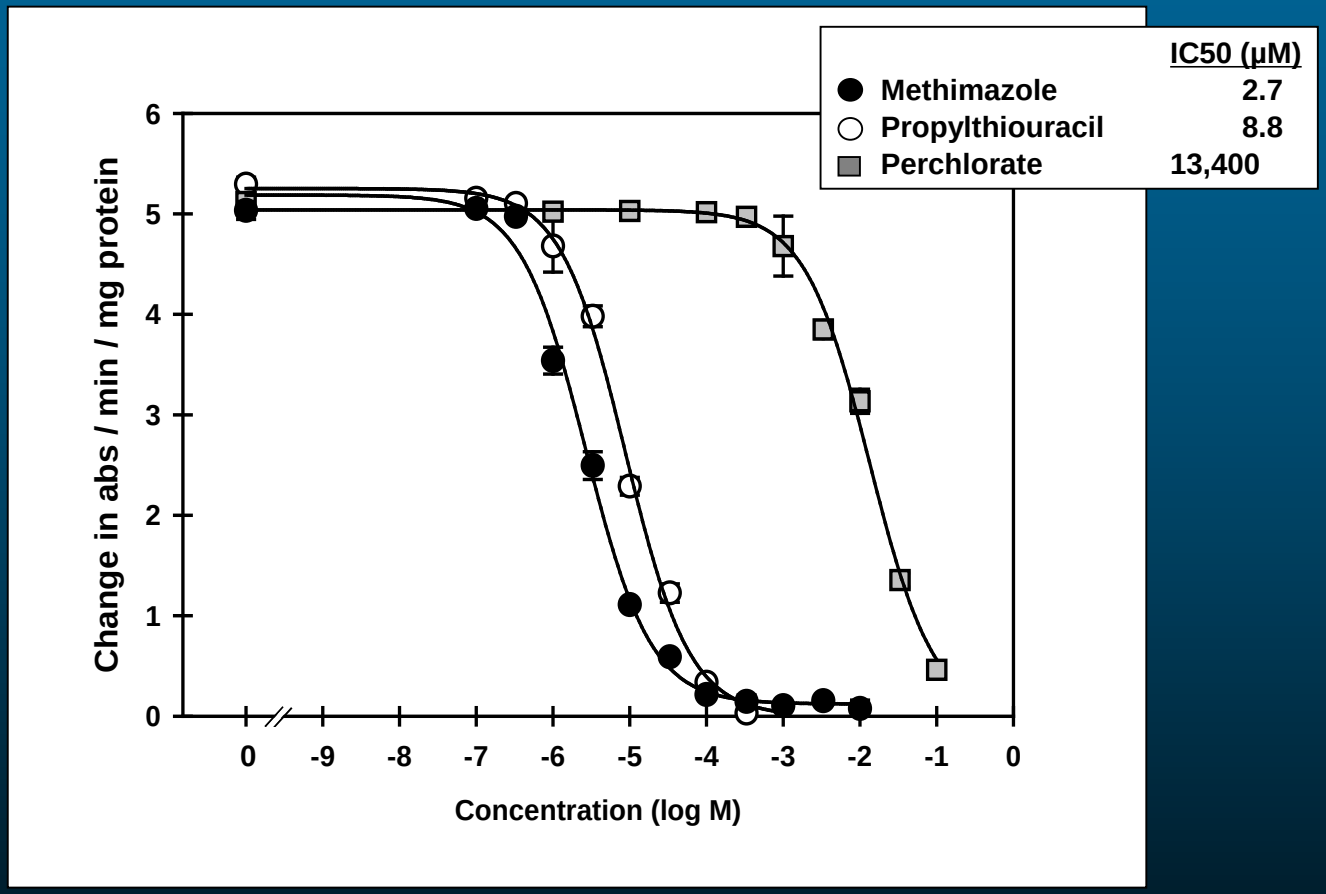

benzoates



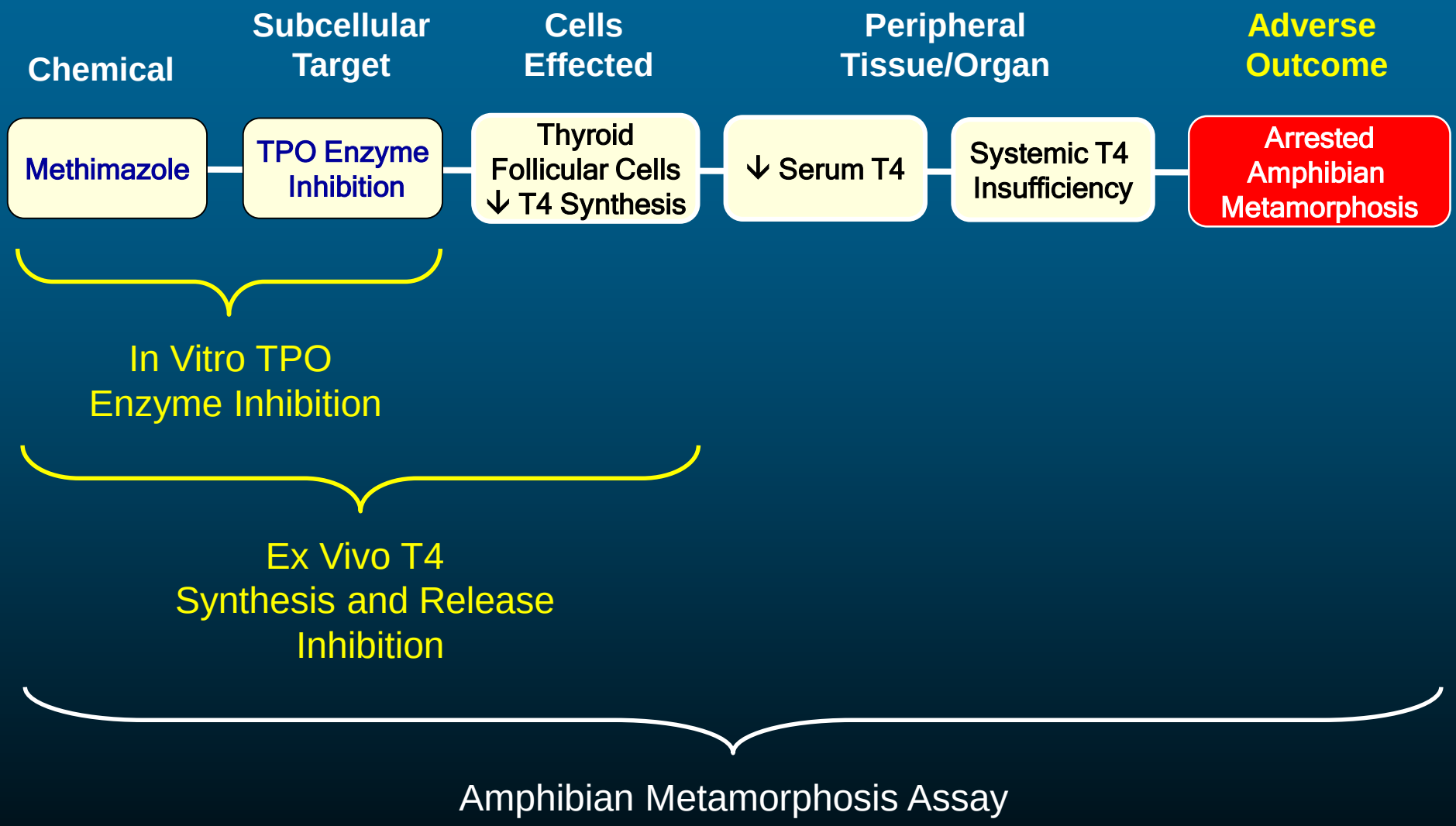

phthalates



# In Vitro TPO Inhibition by Model T4 Synthesis Inhibitors

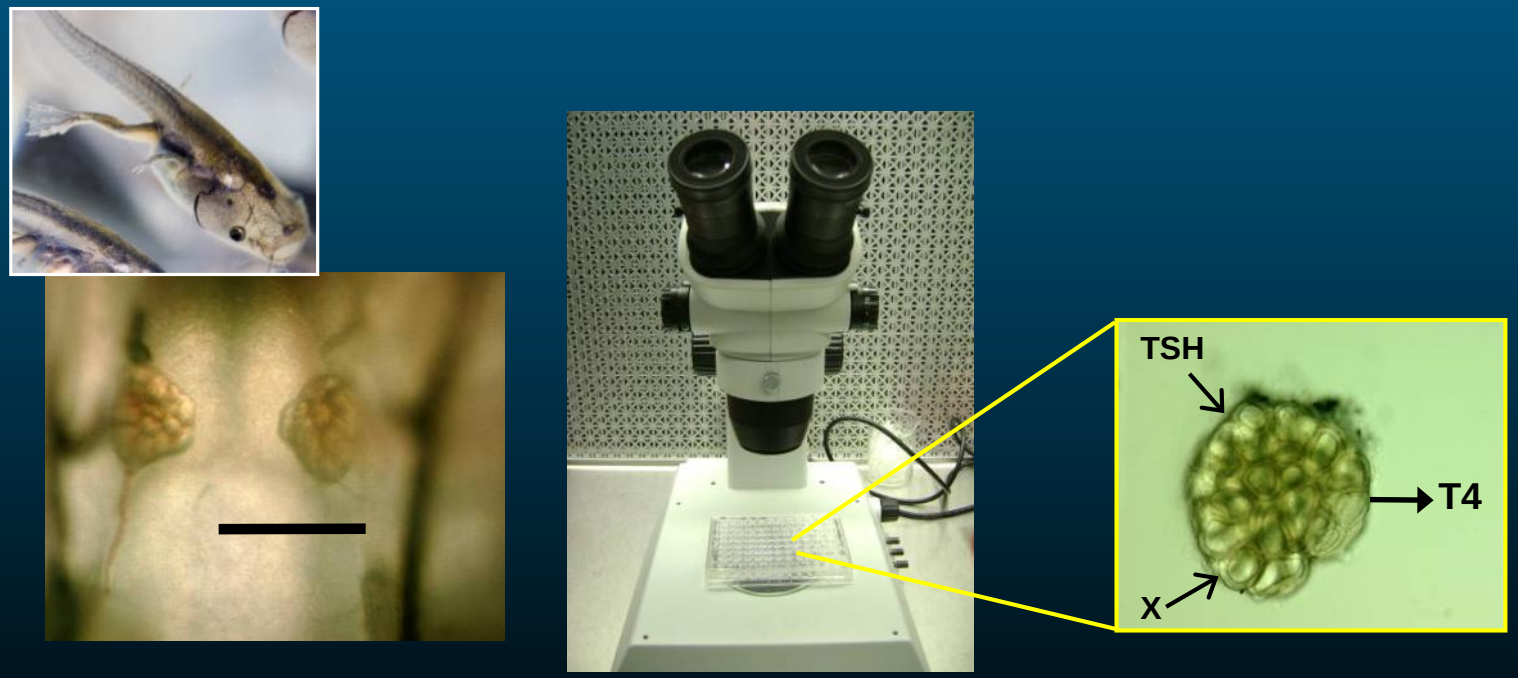


# Tiered Assay Approach from Molecular Initiating Event to Adverse Outcome



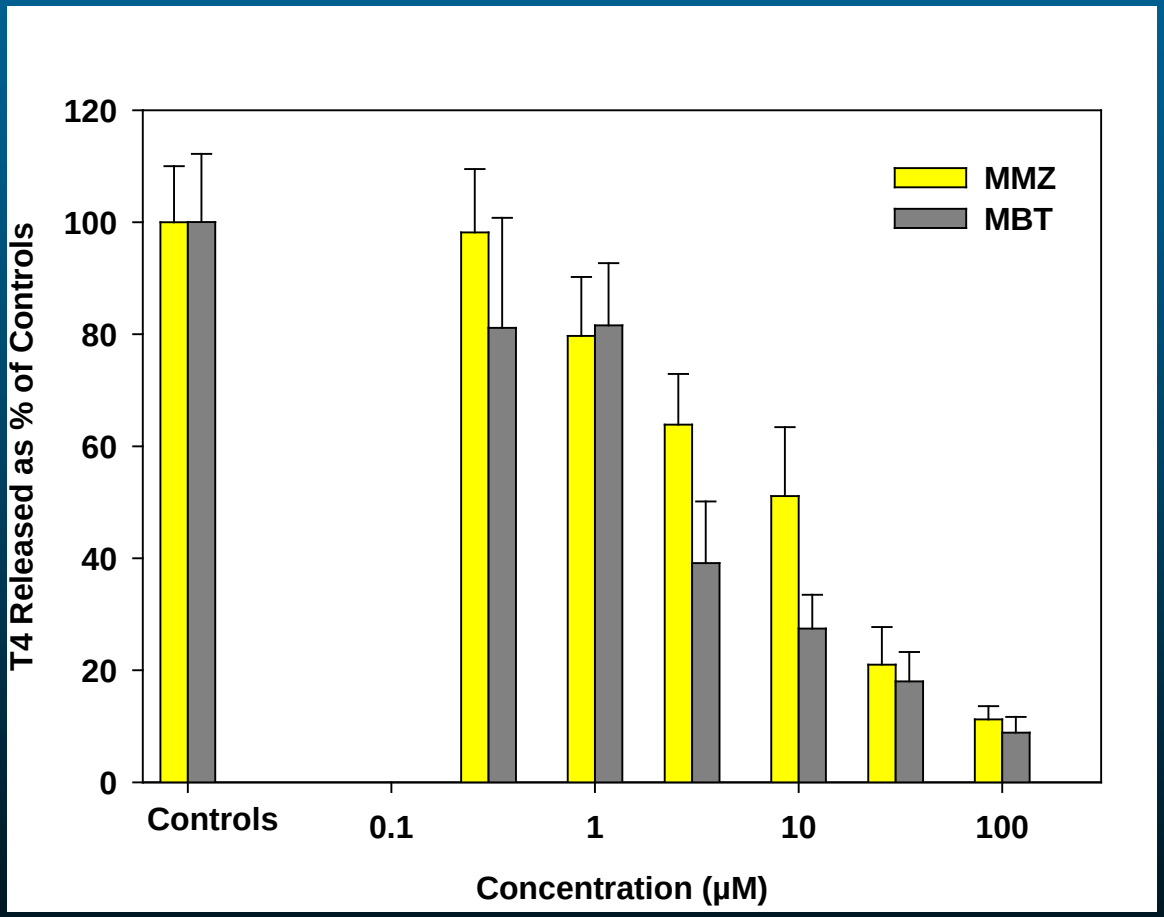
# Ex Vivo Assays: Thyroid Explant Cultures

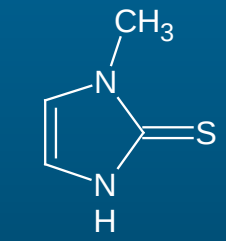
- *X. laevis* explant culture assays
  - Dissect glands from NF stage 59 tadpoles
  - Culture in 96-well plates
  - Inhibition of bTSH stimulated T4 synthesis and release

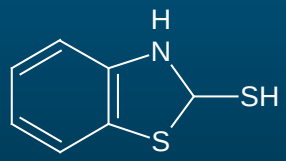


# In Vitro Assays: Thyroid Explant Cultures

- Test chemicals from TPO inhibition assay



# Amphibian Metamorphosis Assay

- OECD 21d AMA protocol
- Initiate at NF Stage 51
- 21d exposure duration
- continuous flow through waterborne exposure

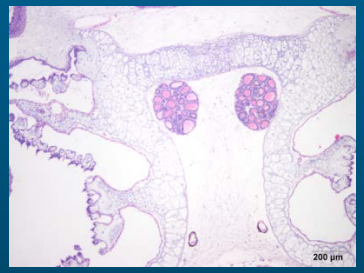
Endpoints

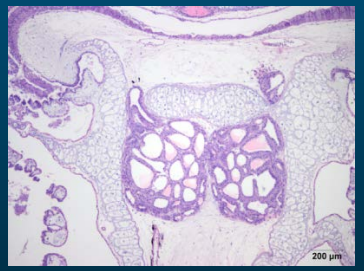
- Metamorphic Development
- Thyroid Histology

- Thyroidal Iodo-amino Acids by HPLC-ICP/MS
- Serum T4 and T3 by HPLC-ICP/MS
- Sodium Iodide Symporter (NIS) mRNA Expression
- Thyroid Stimulating Hormone by ELISA

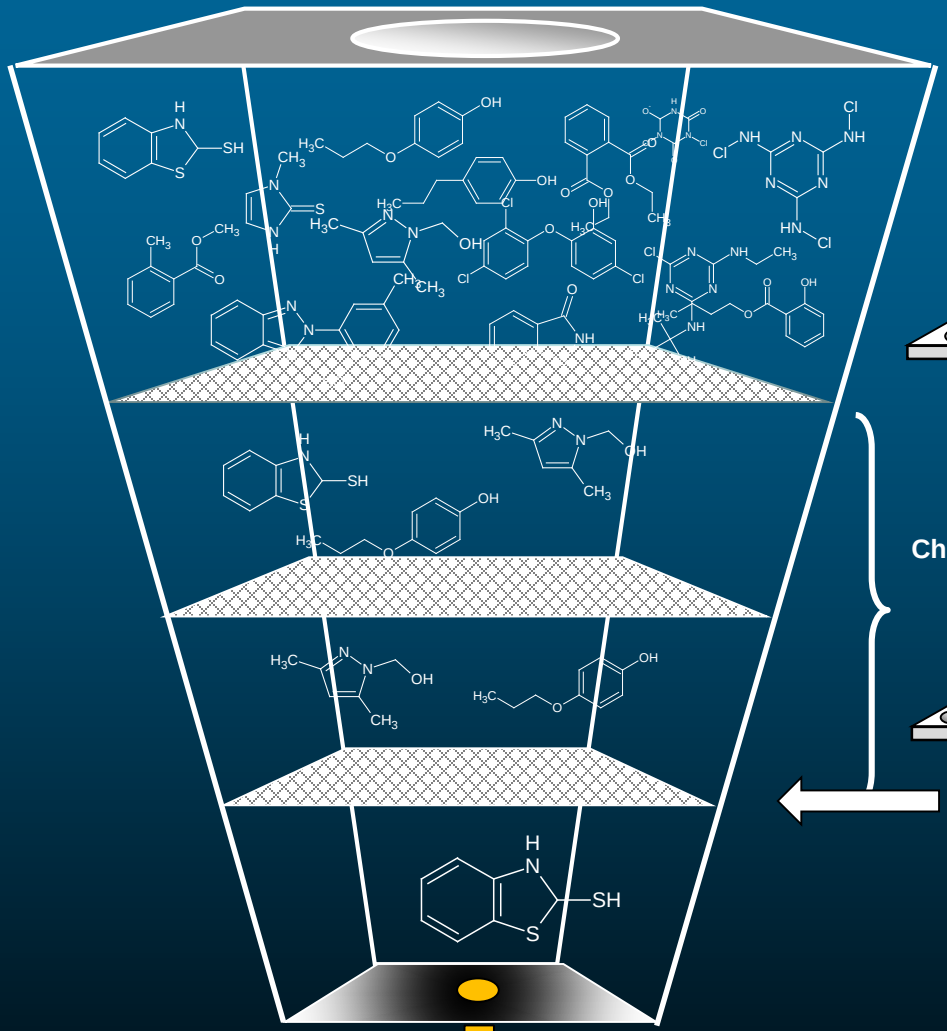
# Verification of Activity in Vivo

| 21 Day MBT Exposure | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [MBT] (µg/L) | | Final NF Stage | | | | | | | | | |
| | | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 |
| 0 | | 0 | 0 | 0 | 0 | 5 | 38 | 10 | 13 | 32 | 2 |
| 23 | | 0 | 0 | 0 | 0 | 14 | 20 | 9 | 32 | 25 | 0 |
| 47 | | 0 | 0 | 0 | 2 | 15 | 36 | 7 | 27 | 14 | 0 |
| 109 | * | 0 | 2 | 2 | 8 | 17 | 30 | 7 | 30 | 5 | 0 |
| 214 | * | 0 | 2 | 8 | 43 | 37 | 8 | 0 | 2 | 0 | 0 |
| 435 | * | 40 | 33 | 22 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |

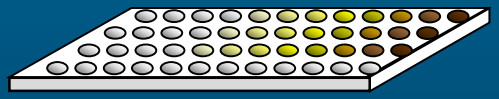


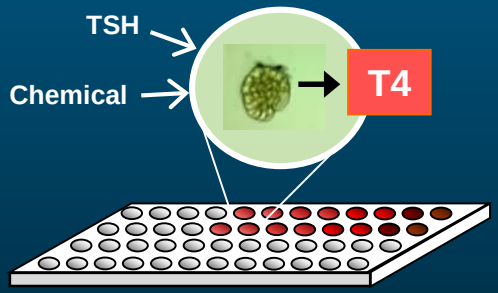



# Tiered Assay Approach to Prioritization of Chemicals & Chemical Classes for Further Testing



Thyroid Peroxidase Inhibition In Vitro






Cytotoxicity Filter

Amphibian Metamorphosis Assay

# Conclusions

Identification of effects-based chemical categories that can affect a given endpoint helps to queue chemicals for targeted testing.

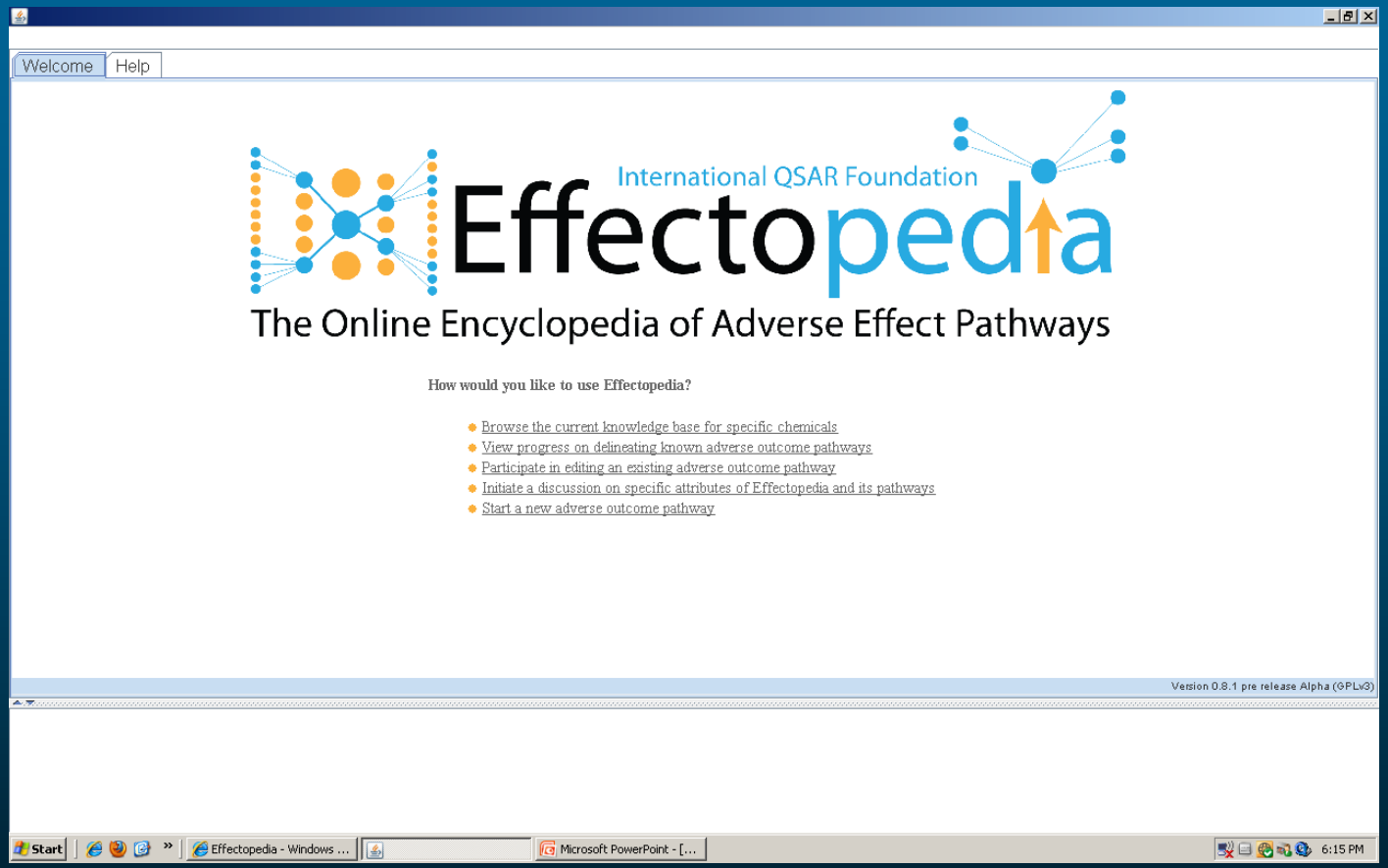
The more we understand what classes of chemicals affect which given pathways will help in directing future toxicity testing requirements.

= Reduced animal usage
= Reduced effort
= Reduced cost

# *Thank you.*

# Effectopedia

## The Knowledge Base for Adverse Outcome Pathways

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